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AIB1, a p160 coactivator, binds to estrogen receptor (ER) and increases estrogen-dependent transcription. It is overexpressed in 64% of primary breast tumors. We hypothesize that genes required for estrogen-dependent growth utilize AIB1 for their transcription. Our hypothesis predicts that decreasing AIB1 should inhibit estrogen-dependent growth and transcription. Our experimental goal was to design inhibitors to AIB1, introduce them into MCF-7 cells, and assay cell proliferation and progesterone receptor (PgR) expression as end-points. We designed two types of inhibitors. The first was a small polypeptide that contained the 11 amimo acids of the HIV TAT protein that have been shown to transduce proteins into cells, and the 19 amino acids from the AIB1 LXXLL box 2 that has been shown to bind to ER. The idea was that this polypeptide would compete with the endogenous, full-length AIB1 for binding to ER and thereby disrupt function. However, this polypeptide had no affect on estrogen-dependent growth or PgR expression. We have created MCF-7 Tet-Off cell lines and are stably transfecting a second inhibitor under a TRE-promoter that contains all three nuclear receptor interaction boxes of the AIB1. Affects on estrogen-dependent growth and transcription in these cells are not yet known for this inhibitor.

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INTRODUCTION

AIB1, a p160 coactivator, binds to estrogen receptor (ER) and increases estrogen-dependent transcription. It is overexpressed in 64% of primary breast tumors. We hypothesize that genes required for estrogen-dependent growth utilize AIB1 for their transcription. Our hypothesis predicts that decreasing AIB1 should inhibit estrogen-dependent growth and transcription. Our experimental goal was to design inhibitors to AIB1, introduce them into MCF-7 cells, and assay cell proliferation and progesterone receptor (PgR) expression as end-points. We designed two types of inhibitors. The first was a small polypeptide that contained the 11 amino acids of the HIV TAT protein that have been shown to transduce proteins into cells, and the 19 amino acids from the AIB1 LXXLL box 2 that has been shown to bind to ER. The idea was that this polypeptide would compete with the endogenous, full-length AIB1 for binding to ER and thereby disrupt function. However, this polypeptide had no affect on estrogen-dependent growth or PgR expression. We have created MCF-7 Tet-Off cell lines and are stably transfecting a second inhibitor under a TRE-promoter that contains all three nuclear receptor interaction boxes of the AIB1. Affects on estrogen-dependent growth and transcription in these cells are not yet known for this inhibitor.

BODY

Our goal was to inhibit AIB1 function in MCF-7 cells to determine if this protein was required for estrogen-dependent cell growth or gene expression.

The first inhibitor we designed was to take advantage of the transduction sequence found in the HIV TAT protein. This small, 11 amino acid sequence, can transduce polypeptides into almost any cell type. Such an inhibitor would be quite versatile. We selected the box 2 LXXLL region of the AIB1 protein to add to the TAT sequence. We chose this fragment of AIB1, because it has the highest binding affinity for ER (1). The total peptide was

YGRKKRRQRRRGQEKHRILHHKLLQNGNSPAE.

This peptide was chemically synthesized and added to the culture media of MCF-7 cells at a final concentration of up to 10 micromolar. Cells were treated with and without 1 nM estradiol and assayed for growth rate and progesterone receptor expression. Estradiol increased the doubling time of the cells from 350 hours to 67 hours in withdrawal media. Addition of the peptide had no affect on this doubling rate. The expression of progesterone receptor was assayed by whole cell uptake assays and western blot. Estradiol strongly induced expression of both isoforms of this protein from undetectable levels in withdrawal media over three days. Addition of the peptide had no affect on this doubling rate.

We verified the functionality of the peptide by assaying for the estradiol dissociation rate from estrogen receptor. Bound peptide was expected to substantially decrease the ligand dissociation rate since it stabilizes helix 12 in a conformation that covers the ligand binding pocket of ER (2). Our peptide had a dramatic effect on the dissociation of 3H-estradiol from the ER protein. The t(1/2) for estradiol on the ER was 34 minutes and was increased to 216 minutes in the presence of 10 micromolar peptide.

Using this assay we determined that the approximate binding affinity of our peptide to the ER in vitro was about 3 micromolar. Our failure to observe any effects of this peptide on estrogen-induced growth and transcription in our MCF-7 cells could be due to several problems; 1) failure to achieve a sufficiently high concentration, 2) degradation of the peptide in the cells, 3) failure of the peptide to localize to the nucleus. We chose a design for a second inhibitor that would address these potential problems.

The second AIB1 inhibitor we have constructed contains the fragment of the AIB1 cDNA from #1965-2489 (175 amino acids) that contains all three receptor interaction domains with LXXLL motifs, but none of the activation domains. The construct also contains the SV40 large T antigen nuclear localization signal, 2 HA antigen tags, and a Kozak sequence for efficient translation. Upon translation this construct will yield a 23Kd protein that will localize to the nucleus and efficiently bind the ER. We have constructed Tet-Off MCF-7 cell lines and are in the process of stably transfecting our construct into these cells under a TRE containing promoter. We chose this design to achieve the highest possible expression of the inhibitor without adding tetracycline to the media. Addition of tetracycline should inhibit expression of the inhibitor. Our future plans are to assay these stable cell lines for estrogen-dependent growth and gene expression in the presence and absence of tetracycline. We will be able to quantitate the expression of our inhibitor by Western blot for the HA tags and verify its nuclear localization by immunocytochemistry for the HA tags.

KEY RESEARCH ACCOMPLISHMENTS

- Characterization of TAT-LXXLL polypeptide as a potential inhibitor of ER-AIB1 interaction in vivo. Functioned to inhibit ligand dissociation in vitro, but did not affect ER function in MCF-7 cells.

- Derived and characterized MCF-7 cell lines stably expressing Tet-Off transcription factor. Cells lines were characterized for estrogen-induced growth and ERE-luciferase expression.

- Construction of construct for stable, repressible expression in MCF-7 cells of a second potential ER-AIB1 interaction inhibitor. Construct contains the receptor interaction domains of the AIB1 protein, 2 HA-tags, nuclear localization signal.

REPORTABLE OUTCOMES

- Development of Tet-off MCF-7 cell lines

- Construction of potential inhibitor of ER-AIB1 interactions

CONCLUSIONS

The development of molecule that can disrupt ER-AIB1 interactions will provide a critical tool to dissect the estrogen-induced pathways that are dependent on this interaction. We have determined that he use of a TAT-peptide is not effective in our system, probably due to the limited concentrations we can achieve in the cell over time. We have turned our efforts to a stable expression system.

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